TABLE 3.
Results of Routine Examination of Samples 21–49.

Sample.	Source.	Macroscopical Characters.						Number of Grains Giving—												Germination Tests.		
		No. Broken Pieces in a Random Sample of 300 Whole Grains.	% Pink Grains.	% Smudged and Black-pointed Grains.	% Shot or Sprung Grain.	% Sound Grain.	Degree of Pinching.**	*Alternaria* spp.	*Epicoccum* sp.	*H. tritici-repentis.*	*Chaetosporium herbarum.*	*Septoria nodorum.*	*Penicillium* spp.	*Cephalosporium* sp.	*H. sativum.*	*H. avenae.*	*Aspergillus* spp.	Bacteria.	Miscellaneous.*	No Organisms.	Germination in Agar.	Germination in Soil.	Number of Seedlings with Lesions.***
21	Gunnedah (Gabo) ..	27	9	8	—	83	2	75	4	15	4	1	—	—	—	—	—	1	—	3	44	75	4
22	Edgeroi (Kendee) ..	—	9	4	—	87	5	59	7	14	17	45	2	1	1	1	—	—	1	—	6	93	3
23	Willala (Gabo) ..	3	8	5	—	87	8	70	4	26	8	5	—	—	—	—	—	1	2	—	33	89	1
24	Gunnedah (Cailloux)	6	15	22	—	63	0	76	6	18	6	—	—	1	—	—	—	4	2	—	11	93	—
25	Gunnedah (Kendee)	14	19	9	—	72	1	78	8	9	4	2	—	—	1	6	1	4	1	—	32	78	—
26	Willala	—	10	7	—	83	8	57	11	39	1	1	—	—	—	—	—	—	—	2	73	96	3
27	Maul's Creek ..	—	19	4	—	77	5	51	3	46	1	—	—	—	—	—	—	—	1	2	66	95	1
28	Gunnedah	18	1	3	—	96	1	93	—	3	2	—	2	—	—	—	—	—	1	2	78	83	—
29	Edgeroi (Celebration)	—	—	—	—	100	2	87	4	2	4	—	—	—	—	—	—	—	—	6	37	87	1
30	Gunnedah (Charter)	—	5	4	—	91	1	50	5	19	6	1	—	—	—	1	—	—	—	—	73	91	1
31	Culgoora (Kendee)	2	4	5	—	91	7	80	8	19	5	—	—	—	2	1	—	—	—	—	32	94	—
32	Baan Ba (Charter) ..	7	1	2	—	97	9	71	2	17	1	—	—	—	—	3	—	—	1	8	50	86	2
33	Griffith	14	—	18	—	82	1	79	6	—	6	—	6	1	—	—	—	—	1	4	86	92	—
34	Binnaway	5	8	6	23	63	2	85	6	3	1	3	27	1	—	—	1	1	—	—	40	88	—
35	Baradine	14	3	21	31	46	2	89	12	2	1	—	3	2	1	—	—	2	—	—	69	68	—
36	Pallamallawa ..	2	6	14	27	53	2	71	20	18	—	—	4	—	4	—	2	—	3	—	33	88	4
37	Parkes	21	2	9	21	68	2	87	8	2	1	—	1	2	—	1	—	—	4	1	64	72	2
38	North Star (Charter)	9	2	17	55	26	2	81	24	13	2	2	1	1	3	—	—	1	1	—	43	80	13
39	Hawkesbury College	—	—	4	—	96	4	21	70	—	4	6	1	1	—	—	—	—	4	1	14	77	2
40	Castle Hill	—	2	3	—	95	4	25	51	—	2	2	1	6	—	—	—	—	4	20	5	36	—
41	Narromine	2	1	2	47	50	3	88	6	4	7	—	3	—	2	—	—	2	3	2	66	81	4
42	Gollan (Kendee) ..	—	1	9	57	33	2	95	9	—	—	—	5	4	—	—	—	3	1	—	52	64	5
43	Armatree	8	2	4	57	94	2	72	12	4	—	—	1	—	—	—	—	—	4	8	47	83	1
44	Yeoval (Kendee) ..	117	1	8	17	74	2	84	13	3	—	1	—	2	—	—	6	—	1	1	31	49	—
45	Coonabarabran ..	12	1	17	28	54	2	86	12	2	—	—	2	2	—	1	—	—	5	—	65	71	1
46	Gravesend	8	13	10	14	63	2	56	4	35	1	8	—	—	—	—	—	—	2	2	74	79	6
47	Trangie	9	2	18	16	64	1	82	9	12	5	—	—	—	1	—	1	—	—	—	75	80	4
48	Finley	23	0·3	2·3	—	97·3	1	52	2	2	6	1	—	—	—	—	3	—	1	38	93	90	—
49	Finley (Pinnacle) ..	4	—	1	—	99	0	61	2	—	4	—	—	—	—	—	1	—	5	31	78	94	—
	Average	11·0	4·9	8·1	11·6	75·4	3·0	71·1	11·3	11·3	3·4	2·7	2·0	0·8	0·5	0·5	0·5	0·7	1·7	4·5	50·6	81·7	2·0

100 grains were used in each germination and plating test.

* *Pullularia pullulans, Podosporiella verticillata* and species of *Trichoderma, Rhizopus, Mucor, Cunninghamella, Botrytis, Nigrospora, Fusarium, Ascochyta,* yeasts and various unidentified fungi.

** The degree of pinching is indicated by allotting each sample a figure from an arbitrary scale of 1–10. The figure for sample 40, which was very badly affected, was fixed at 9.

*** Nearly half these lesions when examined could not be attributed to any parasitic agent. Of the remainder about equal numbers were caused by *H. sativum* and *S. nodorum.*

They may fit *A. peglioni* as described by Curzi (1926) but, except for their shorter chains of conidia, they are similar to *A. tenuis* and could be regarded as extreme types of this very heterogeneous form-species. Simmons (1952), however, warns that the increasingly common practice of treating morphologically similar populations of the Fungi Imperfecti as genetically similar or identical species is based on false premises, in that such similar populations need not necessarily have any great degree of genetic similarity. This is illustrated by his work on three different isolates coming within the modern descriptive limits of *A. tenuis*, each having been derived from an ascospore of a different perfect stage.

Helminthosporium tritici-repentis was found in 34 of the 49 samples, being most abundant in those from the north-west. The percentage of grains from which it was isolated, for any sample in which it occurred, ranged from 2 to 46. This fungus has been recorded in wheat grains only once previously (Galloway, 1936).

A species of *Epicoccum* producing an orange pigment in culture and jet black sporodochia, was isolated from most samples, being obtained from up to 70% of the grains. The highest figures were obtained from samples 39 and 40 from the central coast, the only ones from which *Alternaria* spp. were not the fungi most frequently isolated.

Cladosporium herbarum was isolated from most samples. Frequently it grew out from the brush end of the grain, which suggests that it may not always have been inside the grain but was present deep in the brush and able to withstand the surface sterilization.

Septoria nodorum was found in 22 of the 49 samples, the percentage of grains from which it was isolated in any of these ranging from 1 to 45. It was more abundant in samples from the northern half of the State than the southern, and it is interesting to note that the highest figure was obtained with grain from a crop showing exceptionally bad glume blotch.

Helminthosporium sativum was found in 14 of the samples, highest percentage of grains found to be infected in any sample being 4. The occurrence of *H. avenae*, which was found as frequently as *H. sativum*, is interesting. This is perhaps analogous to the occurrence of *H. avenae* and *H. teres* in wheat grain in Canada (Machacek and Greaney, 1938, Machacek et al., 1951). In inoculation tests the *H. avenae* isolates infected only oats.

Of the fungi isolated, only *H. sativum*, *H. tritici-repentis* and *Septoria nodorum* are known to be actively parasitic on the growing wheat plant. The rest are rarely of any importance though it appears that some may discolour the grain and others, under special circumstances, reduce the viability. *Fusarium* spp. were isolated only very rarely and the isolates were not pathogenic to wheat under glasshouse conditions.

From Table 3 no obvious relation can be seen between the number and nature of the organisms isolated from any sample and the degree of pinching, percentage of smudged grains, percentage of pink grains, or number of grain fragments encountered during the macroscopic examination of 300 whole grains.

When the samples showing no shot or sprung grain and those containing such grain are compared, as in Table 4, no very obvious differences can be observed when the variability within each group is considered. However, it does appear that samples with no shot or sprung grains germinated better and contained more grains from which no organisms were isolated and fewer grains from which *Aspergillus* and *Penicillium* spp. were isolated than those samples with shot or sprung grain. This is to be expected when it is considered that the latter samples must have been subjected to moister conditions than the former. Various authors (Christensen and Gordon, 1948; Laumont and Murat, 1934; Milner et al., 1947*a*, 1947*b*; Thomas, 1937) report observations which indicate that invasion of the grain by such fungi as *Aspergillus* and *Penicillium* spp. is associated with high moisture content and cracking of the epidermis.

From Table 3 it can be seen that, except for the difference pointed out above, germination in agar or in soil bore very little relation to the appearance of the grain or its content of organisms. Germination in agar varied enormously from sample to

sample, often differing considerably from the corresponding germination in soil, usually being considerably smaller. This finding is similar to that of Rosella (1930), who found that the germination of mouchété (smudged) grains, with which *Alternaria* spp. were associated, was lower in agar than in sand.

The reason for the enormous variation between the germinations of different samples in agar, particularly between those whose germinations in soil were much the same, is obscure. It may perhaps be related to the depth of the agar, the attitudes in which the grains were placed therein, the overgrowing of the grain by fungal colonies, or the effect of the surface sterilizing agent.

The number and nature of the lesions developing on the seedlings seemed to bear little relation to the presence, prevalence, or absence in the grain samples of the fungi concerned. If, however, the number of grains infected with these organisms had been higher, no doubt a more clear-cut result would have been obtained. Mead *et al.* (1950) point out that production of lesions was somewhat erratic in their experiments owing to differences in temperature, moisture, and other conditions. Hence, until the optimum conditions for development are known for each organism, it is not clear how far the

TABLE 4.

Derived from Table 3 to Show the Average Populations and Germinations of Samples Containing no Shot or Sprung Grains and Samples Containing 14% or More of such Grains.

Samples.	Germination (%).		Percentage of Grains Giving—							
	In Agar.	In Soil.	*Alternaria* spp.	*Epicoccum* sp.	*H. triticirepentis.*	*S. nodorum.*	*Penicillium* spp.	*Aspergillus* sp.	Miscellaneous.	No Organisms.
Containing no shot or sprung grain	47·7	85·1	74·4	11·4	14·6	3·6	0·7	—	8·3	7·0
Containing shot or sprung grain	55·6	74·5	82·2	11·2	8·5	1·3	4·1	3·6	7·0	0·5

number and nature of the lesions developing in the glasshouse can be relied upon as a guide to the amount of seed-borne disease. The lesions developed mainly on the coleoptiles, and, as all the samples were sown and examined at the same time and under the same conditions, the results are comparative. The only pathogens isolated from these lesions were *Helminthosporium sativum* and *Septoria nodorum*.

Little is known of the importance of *S. nodorum* in grain. Machacek (1945) found lesions on the coleoptiles and first leaves of seedlings developing from infected grains, but it has not been shown that the disease can progress beyond this stage. In this study lesions have been observed on the coleoptiles only, and of all the organisms isolated from surface sterilized grain, *H. sativum* is the only one known to cause a seed-borne disease.

It appears from the literature that although fungi can remain viable within the grain for long periods, many species lose their viability relatively quickly (Machacek and Greaney, 1938; Machacek and Wallace, 1952). As can be seen from Table 5, there was a considerable reduction in the viability of the internal flora in sample 20 over a period of seven months. However, the main species were still viable in many of the grains 13 months after harvest.

As samples 1–20 were examined six months after harvest it seems probable that the internal flora had already undergone a decrease in viability. This may explain why these samples contained a higher percentage of grains from which no organisms were isolated than those of the 1950 harvest.

Distribution of Mycelium within the Grain.

Most work on this subject has been carried out with discoloured grains in which the mycelium has been observed to be most abundant in the pericarp (Bolley, 1910; Curzi, 1926; Fomin and Nemlienko, 1940; Laumont and Murat, 1934; Machacek and Greaney, 1938; Minz, 1943; Rosella, 1930: Weniger, 1935). Where *Alternaria* spp. are the main fungi present the mycelium has usually been found to be restricted to the pericarp, but *H. sativum* has been observed penetrating the embryo and endosperm (Fomin and Nemlienko, 1940; Weniger, 1935).

Dastur (1935), when studying black-pointed grains, with which several organisms were associated, found hyphae in great abundance in the funicle and in the pericarp in the central region of the grain furrow, and observed that they creep between the pericarp and seed coat, where they form a kind of stroma, but were not found in the embryo or endosperm.

Table 5.

A Comparison of the Viability of Organisms within Grain Before and After a Storage Period of Seven Months at Room Temperature.

Time of Plating.	Number of Grains Giving—					
	Alternaria sp.	*H. tritici-repentis.*	*H. sativum.*	Bacteria.	Miscel-laneous.	No Organisms.
June, 1950	52	15	—	4	5	23
January, 1951	16	4	1	2	1	75

Sample 20 used, harvested December, 1949. 100 grains were plated on each occasion.

Bockmann (1933) inoculated wheat and barley seeds with *Cladosporium herbarum*, *Alternaria tenuis* and *A. peglioni* and found that they grew in the pericarp but did not penetrate the testa. He observed hyphae of *C. herbarum* to pass from cell to cell through the wall pits. Rosella (1930) observed hyphae of an *Alternaria* sp. to progress in a similar way.

Curzi (1926) found that the mycelium was not restricted to the discoloured area and that it occurred in clean grains as well, while Laumont and Murat (1934) found hyphae in the pericarp of both clean and discoloured grains belonging to a number of wheat varieties of different origins.

Oxley and Jones are reported by Hyde (1950) to have found mycelium in normal wheat grains in the space between the epidermis and the cross layer, and Hyde found subepidermal fungi in a similar position in normal wheat grains from almost all the wheat growing areas of the world, there being, however, a wide variation in the density of the mycelium and the area of the pericarp invaded.

Hyde quotes Marcus as finding mycelium between the pericarp and testa, growing in particular abundance in the large cavities at the side of the crease, which suggests a similar position, and it may be that Marcus and Dastur (1935) mistook the position of the mycelium.

Christensen (1951) found mycelium between the cell layers of the pericarp, on the inner side of the pericarp, and occasionally within the cells of the pericarp. However, as he peeled strips of "pericarp" from the grain, it is possible that the bulk of the mycelium he observed was actually in the space between the epidermis* and the cross layer, as it is usually the epidermis which is detached where grains are peeled.

* The epidermis according to J. M. Hector, Introduction to the Botany of Field Crops, Vol. I, Johannesburg, 1936, fig. 42, includes all layers outside the cross layer.

In this study, when the epidermis was stripped from grains which had been soaked in hot water for 30–60 minutes, hyphae were observed attached to the inner surface over its whole area in all cases, even in grains from the cleanest samples, whether the grains were discoloured or apparently normal. There was, however, considerable variation in the density of growth which was particularly dense in shot, discoloured grains from sample 44. The mycelium was usually most dense at the tip and along the crease, was branched, septate, usually hyaline, and apparently intercellular.

Examinations of cross sections showed mycelium occurring abundantly between the epidermis and cross layer, being very strongly developed in the large cavities between these layers along the crease, in the funicle and around the embryo. Occasionally hyphae were also observed between the cells of the epidermis.

In no case was any mycelium detected in the endosperm or embryo. However, it is possible that in some cases it was present in these tissues as hand sections only were examined.

TABLE 6.

A Comparison of the Results Obtained with Pink and Apparently Normal Grains from Samples from the 1950–51 Harvest.

Sample.	Pink Grains.						Clean Grains.					
	Number of Grains Plated.	Germination in Agar.	Number of Grains Giving—				Number of Grains Plated.	Germination in Agar.	Number of Grains Giving			
			Alternaria spp.	*H. tritici-repentis.*	Miscel-laneous.	No. Organisms.			*Alternaria* spp.	*H. tritici-repentis.*	Miscel-laneous.	No Organisms.
21	20	9	1	19	1	—	20	8	12	7	—	1
22	20	3	6	14	5	—	20	2	16	2	13	—
23	20	—	4	17	2	—	20	2	13	2	6	—
24	20	4	3	20	2	—	20	6	15	2	4	—
25	20	13	8	13	4	—	20	16	16	1	2	—
26	20	11	2	18	1	—	20	15	15	5	1	—
37	20	13	2	19	3	—	20	13	18	1	3	—
Total ..	140	53	26	120	18	—	140	62	105	20	29	1
Average (%)		37·9	18·6	85·7	12·6	—		44·3	75·0	14·3	20·7	0·7

Investigations with Atypical Grains.

Pinched Grains.

In a test carried out using sample 20, no differences were detected between the microfloras of a group of pinched grains and a group of plump grains.

Mustard Grains.

A small proportion of the grains in certain samples showed a mustard coloration, and in a similar test to the above, using samples 22, 29 and 21, no obvious differences were observed between these grains and those which were apparently normal.

Pink Grains.

It can be seen from Table 6 that, while *Alternaria* spp. were the fungi most commonly isolated from apparently normal grains, *Helminthosporium tritici-repentis* was the main organism isolated from pink grains, being found in 85·7% of these, as opposed to 14·3% of the clean grains. Preliminary tests carried out with samples from the 1949–50 harvest indicated a similar relationship.

This association, supported by the fact that the fungus secretes a pink pigment when grown on agar media and sterile wheat straw, indicates that it is almost certainly responsible for the pink pigmentation of the grain.

When the epidermis was peeled off pink grains it appeared to be more or less colourless, the remainder of the grain being as strongly coloured as before. In transverse sections of the grain the pink colour seemed to be most intense in the aleurone layer and did not extend far into the endosperm. It seems probable that the fungus develops in the pericarp and produces a pigment which is absorbed mainly by the outer layers of the endosperm.

H. tritici-repentis was not isolated from some pink grains. In these cases, the coloration might have been due to some other cause, or the fungus might have been killed by the sterilizing agent, or have been unable to grow out of the grain and compete with the other organisms present. On the other hand, the presence of the fungus in a grain was not always associated with the development of a pink colour.

No other record of the association of *H. tritici-repentis* with a pink grain colour has been found. The most common fungi associated elsewhere seem to be *Fusarium* spp., but Atanasoff (1920) reports that *Alternaria* and *Macrosporium* spp. can cause similar discolorations, while Curzi (1929) records *Acremoniella* sp. associated with reddened caryopses.

The *Epicoccum* sp. so frequently isolated produces an abundance of orange pigment on P.D.A., but no evidence was obtained that it was responsible for any discoloration of the grain. Ito and Iwadare (1934), however, record that *Epicoccum* spp. caused a reddening of rice grains.

H. tritici-repentis has never been recorded as causing a seed-borne disease, or reducing germination. Of 100 pink grains from sample 22, 92 germinated in soil, all seedlings being healthy, while 94 of 100 apparently normal grains germinated under the same conditions. Hence, apart from the discoloration which it might cause, the presence of this fungus is apparently unimportant. There is, of course, the possibility that, with different environmental conditions, germination might be reduced and seedling lesions develop.

Black-pointed Grains.

Numerous plating tests were carried out with black-pointed grains, on samples from different sources, and at various times after harvest. The results showed (*a*) that fungi were isolated more frequently from black-pointed grains than from clean normal grains, and (*b*) that black-pointed grains yielded more *Alternaria* spp. than normal grains. It is clear, nevertheless, that the presence of *Alternaria* spp. in a grain is rarely associated with a discoloration, since the proportion of black-pointed grains in the samples was low, whereas the proportion of grains containing *Alternaria* species was high (Table 3).

Examination of black-pointed grains showed that the cell walls in the dark area were a diffuse dark brown colour, the discoloration being limited to the pericarp and not extending below this into the embryo or endosperm, a finding similar to that of Peyronel (1926). The distribution and density of mycelium in the pericarp of the black-pointed grains was apparently the same as that in clean grains from the same samples.

The literature concerning black-point is in many cases conflicting and inconclusive and so extensive that it cannot be reviewed here. It is known that *Helminthosporium sativum* and *Pseudomonas atrofaciens* can cause it, and that a less clearly defined discoloration may be produced by *Xanthomonas translucens* var. *undulosum*, and it seems that *Alternaria* spp. and perhaps other fungi are often responsible. Also the possibility of a non-parasitic cause must not be overlooked.

The evidence obtained from this study suggests that the type of black-point commonly occurring in New South Wales is associated with species of *Alternaria*, but a more thorough study is needed to establish the cause of this condition.

Factors Affecting the Population Present.

The Surface Flora.

The presence of most organisms, except perhaps for certain bacteria which multiply on the surface, is accidental. The grain is contaminated with air-borne fungi and bacteria and, mainly during harvesting operations, with those growing on the wheat plant.

The Internal Flora.

(*a*) *Air-borne inoculum.*

Machacek and Greaney (1938) and El-Helaly (1947) conclude that, in Manitoba and Egypt respectively, infection of the grains with fungi which cause black-point arises from air-borne spores which are usually deposited in the largest numbers about the time the kernels are maturing. During the period 1932–36, Machacek and Greaney found large numbers of *Alternaria* spores in the air at this time, whereas those of *Helminthosporium sativum* occurred in an appreciable quantity only in 1935; but nevertheless, *Alternaria* spores were always found to outnumber these by at least four times.

Spores of a *Helminthosporium* similar to those of *H. tritici-repentis*, *H. sativum*, *Epicoccum*, *Alternaria*, *Stemphylium*, and *Cladosporium*, together with uredospores and numerous unidentified spores, were found on slides exposed in the north-west of the State in November, 1950, so it seems probable that infection of wheat grains in New South Wales also arises largely from air-borne inoculum, and that the fungi which are not actively parasitic enter the grains as they mature.

(*b*) *Mutual relations between micro-organisms.*

Machacek and Greaney (1938) found that over the period 1931–34 *Alternaria* spp. were the predominating fungi in discoloured kernels. In 1935, however, most of the discoloured wheat kernels in the samples examined were found to be infected with *H. sativum*, while *Alternaria* spp. were recovered much less frequently than in previous years. As there was no shortage of inoculum of *Alternaria* spp., it was suggested that an antagonism between the two fungi in the seed was responsible for this, and the hypothesis was supported by the fact that such an antagonism was observed in agar plate cultures.

That such effects are important has been demonstrated further by Niethammer (1938). She found that when *Penicillium expansum* and *Cladosporium herbarum* were simultaneously inoculated into wheat seed grain, the latter gains the upper hand and rapidly suppresses the former. In the case of *C. herbarum* and *Trichoderma koningi*, the latter was found to be the more active.

In the present work numerous antibioses have been observed in agar plates, although, as the colonies were usually some distance apart, some of the less pronounced effects may have been overlooked. *Penicillium* spp., *Trichoderma* sp., *Rhizopus nigricans*, *Mucor* sp., *Botrytis* sp., *Fusarium* sp., one colony of *H. sativum*, and certain bacteria were observed to have a detrimental effect on *Alternaria* spp. and most other fungi. It is not known whether the position is the same within the wheat grain.

Pullularia pullulans and *Epicoccum* sp. showed a very weak antibiosis to *Alternaria* spp. In the case of *Epicoccum* sp. it is possible that this was responsible for the relative freedom of samples 39 and 40 from *Alternaria* spp., although other factors could have been operating.

(*c*) *Amount of disease in crop concerned.*

With active parasites such as *Septoria nodorum*, *Fusarium* sp., and certain bacteria, it seems certain that infection is brought about by these organisms developing on the crop concerned. The same may be true, at least in some cases, for the *Helminthosporium* spp., if only in that amount of disease in the crop influences the concentration of spores in the air in the immediate vicinity.

(*d*) *Climatic conditions.*

El-Helaly (1947) claims that an outbreak of black-point depends, amongst other things, on atmospheric humidity and rainfall, while Adam (1950) noted that a wet spell

after flowering seemed associated with an outbreak. Hyde (1950) found a correlation between the amount of subepidermal mycelium and the atmospheric humidity during the ripening of the grain.

Greaney and Machacek (1946) found in Manitoba that if climatic conditions favoured early ripening and harvesting, the incidence of seed pathogens was low, but if warm, humid weather occurred during ripening and harvesting the incidence of infection was high.

Undoubtedly climatic conditions have a similar influence here. After the 1949 and 1950 samples, which had ripened and been harvested under exceptionally humid conditions, had been examined, it was supposed that in a drier season more of the grain would be free of internal fungous infection, and that of the fungi present a smaller proportion would be pathogens. This supposition was supported by the fact that samples 48 and 49, from Finley, which had ripened under comparatively dry conditions, were very clean and bright, contained the largest percentages of grain from which fungi were not isolated, and were almost free, internally, of pathogenic species.

A brief examination of samples from the 1951 harvest has confirmed this supposition. Conditions during ripening and harvesting were abnormally hot and dry and microscopic and plating tests revealed that only occasional grains contained fungi, these almost invariably being *Alternaria* spp. The mycelium beneath the epidermis, where present, was sparse and confined to the regions over the embryo and at the brush end. The surface flora of the samples examined consisted almost entirely of bacteria and the grains carried very few mould spores.

(*e*) *Conditions after harvest.*

Investigations overseas have shown that *Aspergillus* spp., *Penicillium* spp., and certain other common moulds do not enter sound wheat stored under normal conditions, but enter when the epidermis is cracked, as sometimes occurs in the course of threshing operations, or when the grain is stored with a high moisture content. Milner et al. (1947*a*, 1947*b*) found *Alternaria* to be the main fungus in sound samples, but that *Aspergillus* and *Penicillium* spp. were predominant in samples from the same lots stored with a high moisture content.

Economic Importance.

The organisms occurring on or in wheat grains may be important because:

(i) They cause discolorations which may lower the commercial value of the grain;

(ii) they cause damage during storage, particularly species of *Aspergillus* and *Penicillium*, which cause heating when grain is stored with a high moisture content (Christensen and Gordon, 1948; Hyde, 1950); and

(iii) they may affect the yield and quality of the subsequent crop.

The general opinion in the literature seems to be that while certain organisms, particularly *Helminthosporium sativum* and *Fusarium* spp., may reduce germination and seedling emergence, and cause root rots, *Alternaria* spp. have no effect, and, from the standpoint of seed-borne disease, are not considered important (Brentzel, 1944; El-Helaly, 1947; Fomin and Nemlienko, 1940; Greaney and Machacek, 1942, 1946; Henry, 1923; Machacek and Greaney, 1938; Minz, 1943; Rosella, 1930; Ziling, 1932). There have been two reports in New South Wales of reduced germination in black-pointed grain (Anon, 1939; Stening, 1935), but it is interesting to note from the work of Russell and Ledingham (1941) and Waldron (1936) that a reduced emergence is not necessarily followed by a reduced yield.

Although seed-borne pathogens were rarely detected in the samples used in this study, it is quite possible that there may be important variations in the grain microflora in future years. Also, it is clear that the value of a sample for seed cannot be assessed from its appearance, for a badly discoloured sample may carry less pathogenic organisms and germinate better than an apparently clean one.

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APPENDIX.

Methods of Isolation.

Before adopting the procedure recommended by Greaney and Machacek (1946), tests were carried out to determine the effect of time and nature of surface sterilization, and the nature of the agar medium on the number and nature of the organisms isolated. In each test the germination in agar was also recorded. Platings in the first two tests were made in P.D.A. An untreated control was included in each test. Grain used was from samples 20 and 22.

1. *Time of treatment with alcoholic mercuric chloride solution.*

Times of treatment were as follows: 5 sec., 10 sec., 15 sec., 30 sec., 1 min., 2 min., 3 min., 4 min., 15 min., 30 min., 1 hr., 3 hr., 6 hr., 12 hr., 24 hr.

Bacteria, *Aspergillus*, *Penicillium*, and *Cephalosporium* were virtually eliminated after five seconds' treatment, indicating that they were occurring largely as surface contaminants. As the time of treatment increased beyond 30 seconds, the other organisms continued to be isolated in the same proportions, although the total number of fungi, the number of grains giving organisms, and the germination gradually declined when times of treatment extended beyond four minutes. It was considered, therefore, that nothing would be gained by departing from the recommended sterilization time of four minutes.

2. *Method of sterilization.*

Grain was treated as follows:

(i) Immersed for ten minutes in calcium hypochlorite;

(ii) Immersed for four minutes in alcoholic mercuric chloride solution, followed by washing three times in sterile water;

(iii) As (ii), but without subsequent washing.

Very much the same results were obtained with these three methods, both as to the number and nature of the organisms isolated, and the germination in agar.

3. *Nature of agar medium.*

The media used were malt agar, nutrient agar, and P.D.A. at pH 5–6 and 7–8. Again, very little difference was detected.

After the above tests, it was considered that the sterilization procedure of Greaney and Machacek (*loc. cit.*) was satisfactory, and was followed in all the subsequent work.

When the study was completed the work of Oelofsen* came to the notice of the writers. He concluded that the fungi which grew from kernels treated by methods similar to that used here "were so numerous and so different in nature that they could not possibly all have been borne internally by the seed". The number of fungi isolated was much smaller and fewer genera were encountered when the suture was opened before sterilization, a measure which is claimed to give the mercuric chloride better

* O. N. Oelofsen, Investigation of Cereal Diseases in the Western Cape Province, Science Bulletin No. 289, Department of Agriculure, Stellenbosch-Elsenburg, 1950.

access to the fungi lodged in the groove. He stated that the fact that the pericarp is torn as a result of the treatment makes no appreciable difference to the effect of the disinfectant. He soaked the grains for 12 hours in water before opening the suture, and also removed the embryo.

When grains from local samples were treated without removal of the embryo, by Oelofsen's method, there was, as in his work, a marked reduction in the number of the fungal colonies isolated.

However, as very often a large proportion of the internal mycelium occurs in the cavities between the epidermis and cross layer along either side of the base of the suture and above the embryo, it is felt that Oelofsen's treatment must have some effect on the subepidermal fungi.

YELLOW SPOT DISEASE OF WHEAT IN AUSTRALIA.

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(Plate xii; one Text-figure.)

[Read 26th November, 1952.]

Synopsis.

Yellow Spot of wheat is recorded from New South Wales and Queensland. The symptoms, importance and distribution of the disease, and the morphology of the causal organism are discussed. Pathogenicity tests are reported, and, after an examination of the literature, it is concluded that the fungus concerned is *Helminthosporium tritici-repentis* Died. with the perfect stage *Pyrenophora tritici-repentis* (Died.) Drechs.

Introduction.

Recently a species of *Helminthosporium*, differing markedly from any previously described on wheat in Australia, has been found causing a disease of wheat in New South Wales and southern Queensland. It also differs considerably from all the species described on wheat except *H. tritici-repentis* Died. and *H. tritici-vulgaris* Nisikado.

Although the fungus was isolated from grains of the 1949 harvest, it was not until August, 1950, that infected plants were recognized in the field. These were observed near Gunnedah, and since then the disease has been found to be widespread in the northern half of New South Wales and has been recorded on specimens from southern Queensland.

It seems probable that this disease has been present for some time but has remained inconspicuous until the 1949 and 1950 seasons, which were unusually wet and favoured its spread and development.

Symptoms.

The disease develops mainly on the leaves. The lesions in the early stages are small oval to oblong spots, which gradually increase in size, becoming light brown to dark brown with a yellowish zone at the margin (Plate xii, 1), and may easily be confused with lesions caused by *Septoria nodorum*. The lesions are usually small, but may reach a length of 1–2 cm., although they are rarely more than 0·5 cm. wide, sometimes becoming irregular owing to the coalescence of several spots. Severely attacked leaves wither from the tips, and conidiophores and conidia are produced on the dead tissue. Small black sclerotia frequently develop, particularly on the base of the stem and the sheathing leaf bases.

The presence of the fungus within the grain is often associated with a pink pigmentation, and it seems to have been responsible for almost all of the pink or reddish discolorations observed in New South Wales in 1949 and 1950 (Shaw and Valder, 1952). A similar pigmentation has occasionally been observed on dead glume, leaf and stem tissue.

Characteristics of the Causal Fungus.

1. *Culture.*

On P.D.A. the fungus gives rise to pale grey, low-growing, cottony, non-sporing colonies, sometimes producing deposits of orange and pink pigment in the medium. These may occur uniformly over the whole colony, in rings, or only where the mycelium runs up against the edge of another colony.

As the colonies age, they darken, becoming almost black at the surface of the agar, although the aerial mycelium remains grey. The submerged mycelium shows an abundance of anastomoses, and some of the cells involved in these hyphal fusions swell

* Work undertaken while a student in the Faculty of Agriculture, University of Sydney.

into sub-globose bodies similar to those described by Drechsler (1923) for various species of *Helminthosporium*. In plate cultures these are found in abundance near the lower surface of the agar. Frequently certain of these develop into small black sclerotia, either scattered throughout the colony or aggregated where two colonies have met.

Where the mycelium meets the glass wall of a Petri dish or culture tube, numerous grey fascicle-like structures, usually 1–3 mm. but up to 5 mm. high, and reminiscent of minute bracket fungi, develop. Luttrell (1951) in describing the cultural characters of *H. dictyoides* states that a fringe of tiny, erect clavae, composed of compacted hyphae, develop around the margin of the colony where it comes in contact with the walls of the culture tube. Apart from this record there appears to be no mention of such structures in the literature concerning the genus *Helminthosporium*. However, the writers have observed them in cultures of other species, though they were not formed as abundantly as in cultures of the fungus from wheat.

Drechsler (1923) and Conners (1940) report no conidia in culture for *H. tritici-repentis*, while Mitra (1934) states that this species spored in a day or two when mycelium with a little agar from culture was incubated in a moist chamber, and when it was grown on sterilized straw. Nisikado (1929) reports conidial production by *H. tritici-vulgaris* when it was grown on Beijerinck's or plain agar. Local isolates produced no conidia under any of these conditions, nor on nutrient, malt or maize meal agars.

Cultures that have been kept for some time seem to lose their ability to form sclerotia and clavae, become paler, and often produce more pink or orange pigment than when they were first isolated.

2. *Occurrence of the Perfect Stage.*

The perfect stage of the local fungus was first observed in a culture obtained from grain of the 1949 harvest in which, after some weeks, perithecia of the type described by Wehmeyer (1949) for *Pleospora trichostoma* (Fr.) Ces. and de Not. matured. Single ascospore cultures were obtained and shown to be pathogenic to wheat on which the conidia were later produced. Perithecia, however, have not been observed in culture since.

In the springs of 1950 and 1951, perithecia were observed to be abundant on stubble in the north-west of New South Wales, frequently intermingled with pycnidia of *Septoria nodorum*.

3. *Morphology.*

a. Conidiophores.—The conidiophores are unbranched, dark brown, have a swollen basal cell and occasionally show geniculations (Text-fig. 1, E). Those from leaves collected in the field measured 75–270 × 8–10 μ with 3–11 septa (20 measured). Those produced on leaves kept in a humid atmosphere were somewhat paler and measured 190–280 × 10–11·5 μ with 2–7 septa (20 measured).

Andrews and Klomparens (1952) and Sprague (1950) state that the conidiophores of *H. tritici-vulgaris* show a characteristic light colour towards their tips. This has been observed with local collections, particularly when the conidiophores are produced on leaves kept in a humid atmosphere.

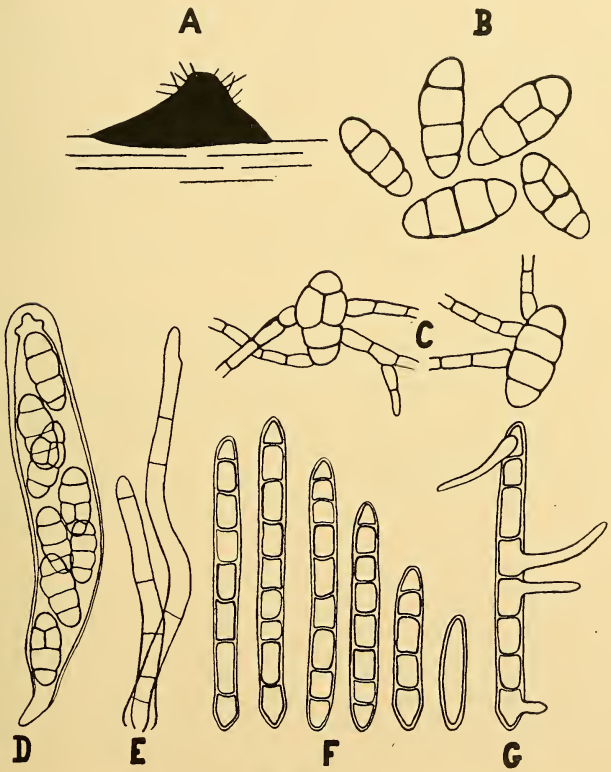
b. Conidia.—The conidia from leaves collected in the field were pale brown, pale olivaceous or subhyaline, thin walled, straight or occasionally slightly curved, more or less cylindrical, often tapering a little towards the distal end, the distal cell hemispherical or hemiellipsoidal, the proximal (basal) cell bearing an inconspicuous hilum and often characteristic in shape, there being a slight constriction at the septum with the basal portion of the cell tapering abruptly in the manner of a cone (Plate xii, 5; Text-fig. 1, F). This shape suggested the appearance of the head of a snake as described for *H. tritici-repentis* by Drechsler (1923).

Drechsler notes that departures from this type are not infrequent, and the same holds for the local fungus (Plate xii, 4). Cultures derived from single spores showing

and not showing the "snake's head" appeared identical, were all pathogenic, and all produced both types of conidia when inoculated on to the host.

Conidia produced under natural conditions in the field measured 23–307 × 13–26 μ (mean 116 × 18·3 μ) with 0–13 (most frequently 6) septa (50 measured). Only five conidia were longer than 156μ.

Conidia produced on diseased tissue kept in a humid atmosphere were subhyaline, straight and cylindrical, with the basal cell often exhibiting, either weakly or strongly, the "snake's head" appearance, and measured 52–216 × 11·5–23 μ (mean 160 × 15 μ) with 0–9 (most frequently 7) septa (50 measured).



Text-figure 1.

A. Sclerotioid perithecium of *P. tritici-repentis* showing setae in the neck region. × 50. B. Mature ascospores. × 350. C. Germinating ascospores. × 350. D. Immature ascus. × 350. E. Conidiophores. × 350. F. conidia. × 350. G. Germinating conidium. × 350.

Secondary conidia were sometimes produced from the tips of the primary ones when diseased tissue was held in a humid atmosphere.

In general appearance, the conidia with the "snake's head" character resemble very much those figured by Drechsler (1923), Mitra (1934) and Dennis and Wakefield (1946) for *H. tritici-repentis*, and those not showing the "snake's head" appearance resemble those figured by Nisikado (1929) and Andrews and Klomparens (1952) for *H. tritici-vulgaris* and by Drechsler (1923) for *H. bromi*.

The conidia germinate from any of their cells (Text-fig. 1, G) and frequently show anastomoses between the germ tubes. When two germinating conidia lie side by side, pairs of germ tubes are proliferated from approximately opposite positions and immediately anastomose giving rise to scalariform figures (Plate xii, 5). A similar phenomenon is described by Drechsler (1923) for *H. bromi*, Mitra (1934) for *H. tritici-repentis* and Graham (1935) for *H. gramineum*.

c. Perithecia.—The development of sclerotioid perithecia of the *Pleospora trichostoma* type has been well described by Drechsler (1923), Wehmeyer (1949), and Webster (1951). When mature the perithecia develop a conical beak (occasionally two) and the beak and other portions of the wall bear stiff black hairs (Plate xii, 2; Text-fig. 1, A).

The asci (Plate xii, 3; Text-fig. 1, D) show an internal annular thickening at the top and each contains eight irregularly biseriate, pale olivaceous ascospores, each with three transverse septa and often with one vertical septum in the second or third cell, there usually being constrictions at the septa (Text-fig. 1, B). These ascospores germinate readily in tap water from either the central or end cells or both (Text-fig. 1, C).

The perithecia measured 0·4–0·7 × 0·25–0·4 mm. (20 measured) and the asci 80–310 × 37–53 μ (mean 245 × 41 μ) (20 measured). Ascospores from different sources varied considerably in size, the difference no doubt being due to differences in maturity, environment and perhaps to variation within the fungus itself. Those which matured in culture were the smallest observed, measuring 42–49 × 14–18 μ (20 measured), while those collected in the field measured 42–65 × 14–30 μ (50 measured).

4. *Tests of Pathogenicity.*

Owing to the fact that no spores were produced in pure culture, mycelium taken from young cultures, and macerated in water, was used as inoculum. After inoculation, the plants were held in a humid atmosphere for 48 hours.

Plants inoculated were seedlings of "Federation" wheat, "Kinver" barley, "Algerian" oats, "Black Winter" (open-pollinated) rye, "Milo" sorghum, "Fitzroy" maize and plants of *Agropyron repens*, *Bromus inermis*, *Cynodon dactylon*, *Elymus canadensis*, *Hordeum leporinum* and *Lolium multiflorum*.

A heavy infection was obtained on wheat and *Elymus canadensis*, a moderate infection on *Agropyron repens* and minute lesions only on *Bromus inermis*. The fungus was re-isolated from all these plants. No infection was obtained on any of the others.

Identification of the Causal Fungus.

Drechsler (1923) reviewed the early history of *H. tritici-repentis* and related species and transferred their perfect stages from *Pleospora* to *Pyrenophora*. He stated (1934) that excessive emphasis on the presence or absence of setose outgrowths had obscured the important differences between these genera and that, rehabilitated as a natural genus through the elevation of *Chaetoplea* to generic rank, *Pyrenophora* again conformed to Fuckel's definition, being properly reserved for the hard sclerotioid perithecial forms having their asexual stages in the *Helminthosporium* series with indiscriminate germination. Until recently this decision of Drechsler's seems to have been followed.

Nisikado (1929) described *H. tritici-vulgaris* on wheat in Japan. It apparently differed from *H. tritici-repentis* in its ability to sporulate easily in culture and in the shape of the basal cells of the conidia, which did not present the "snake's head" appearance. *H. tritici-vulgaris* has since been recorded by Raabe (1937) in Germany, and Barrus (1942), Johnson (1942), Miller (1947), Sprague (1950), and Andrews and Klomparens (1952) in the United States. This fungus has only been recorded on wheat and no cross inoculation tests with grasses are reported.

Sprague (1950) and Weiss (1950) record *H. tritici-repentis* on wheat, rye and numerous grasses in the United States.

Garbowski (1932) recorded *H. tritici-repentis* on rye in Poland, and after preliminary reports (McRae, 1932, 1933; Mitra, 1931), Mitra (1934) described it as it occurred on